B cells in rheumatoid arthritis

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Abstract

Though its etiology remains unknown thus far, the role that autoimmune processes play in rheumatoid arthritis (RA) pathogenesis has been widely proven. Given the easier accessibility of humoral components, the first feature of this contribution to be recognized has been the occurrence of the so-called rheumatoid factor in a large proportion of RA patients. This antibody recognizes the Fc portion of human IgG. By investigating RA pathologic processes and also through experimental models where immune complexes play a fundamental role, many other autoantibodies have then come to our knowledge to be associated with the disease. Their presence and persistence implies that clones of autoreactive B cells survive and proliferate in RA patients under a continuous stimulation. Whether this is a mechanism of disease initiation or just an epiphenomenon is still unclear but no doubt exists that autoantibodies represent a very useful tool in both diagnostic and prognostic terms. Being much more than simple autoantibody producers, B cells are able to secrete many important cytokines and to efficiently present antigens to T lymphocytes in the synovial environment. All of these functions are essential in the development of RA, and lately have claimed attention as B cell depletion has become a common and effective strategy of treatment in RA.

Keywords: Rheumatoid arthritis; B lymphocytes; Autoantibodies; Lymphoid neogenesis

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1. Introduction

Rheumatoid arthritis (RA) is a chronic disorder that mainly targets the synovial membrane of diarthrodial joints but that can also have systemic manifestations [1]. Although considered for a long time a T cell/macrophage-driven pathology, introduction of efficacious B cell-targeted therapies with the use of an anti-CD20 monoclonal antibody (Rituximab) [2] has re-opened the question of B cell relevance in RA pathogenesis also thanks to new acquisitions in both autoantibody-related and autoantibody-independent fields of research.

2. Autoantibody production

Many alterations in the physiologic process of B cell tolerance have been described in RA. Normally, B cells bearing receptors reacting with self-components are counterselected at tolerance checkpoints both in bone marrow and in the periphery through pathways that involve receptor editing, apoptosis induction and anergy. It has been shown that RA patients display a defective B cell tolerance in more than one of these mechanisms [3]. Elevated BLyS levels are found in sera or in synovial fluid of patients with RA and BLyS may promote the inappropriate survival of B cell clones [4]. Synovial production by terminally differentiated plasma cells of both rheumatoid factor(s) (RF) and anti-citrullinated proteins (aCP) has been described together with the demonstration of clonal expansion, somatic mutation and affinity maturation in ectopic synovial germinal center (GC)-like structures [5].

Indirect proof of the incongruous activation of B lymphocytes in RA and of a persistent B cell receptor (BCR) engagement is also the occurrence of malignant transformation as shown by an increased proportion of diffuse large B cell lymphomas (DLBCLs) in RA patients. Though the mechanisms are not clear and likely multiple, this alteration is mediated predominantly by an increased occurrence of the non-GC subtype of DLBCLs emphasizing the role of activated peripheral B cells [6]. Under these circumstances, B cells are pulled to produce a variable set of autoantibodies (autoAbs). Their detection up to years before the disease onset as for RF and aCP again suggests that alterations in the B cell compartment occur at an early stage of RA development [3].

Accumulation of genetic susceptibility and environmental factors is also necessary for mounting this self-reacting response and many HLA association have been recognized [7]. Moreover, a haplotype of the gene encoding the citrullinating enzymes peptidylarginine deiminase (PAD) 4 was shown to be linked to RA, though this association is highly susceptible to ethnic differences [8].

Even if a strong contribution of autoAbs has been elegantly demonstrated in the pathogenic mechanism of experimental models, e.g. anti-collagen II antibodies and aCP in collagen-induced arthritis and anti-glucose-6-phosphate isomerase in the K/BxN model, their role is far from clear in the human disease. Main concerns are that RA develops also in subjects that never come to display any autoAb reactivity and that some of these autoAbs are not disease-specific [1,8].

3. Main autoantibody systems: genesis of RF and aCP

RF binds the Fc portion of human IgG. It is found in up to 80% of RA patients but it is quite unspecific as it is detected in other autoimmune diseases, systemic infections, and in up to 10% of healthy subjects [9]. Nevertheless many differences exist between RF in health and disease. The former is an IgM produced by B1 cells as a “natural antibody” and shows polyreactivity and low affinity. RA-RF instead undergoes Ig-associated gene rearrangement and isotype switching as consequence of affinity maturation and somatic hypermutation that RA B lymphocytes experience under Th and synovial fibroblasts supervision [1]. The nature of its binding to IgG-Fc involves an unconventional part of the BCR leaving space for another (auto)antigen to be internalized, processed and presented to T cells [9]. In support of this hypothesis comes the ability of RF+ B cells to be efficient antigen-presenting cells (APCs) [10] and the demonstration that chromatin-containing immune complexes stimulate RF+ B cells synergistically engaging the BCR and the innate immunity Toll-like receptors (TLRs) [11]. This last finding is of striking interest because it underscores the fundamental contribution of innate immunity to activation of the adaptive response in an autoimmune setting.

Citrullination is the critical step for the recognition of a bunch of proteins (fibrin, vimentin, fibronectin, collagen type II), highly expressed in the synovial environment during inflammation, by a group of autoAbs that specifically occur in RA and referred as aCP [8]. Anticitrulline immunity has recently offered evidences for a very interesting etiologic model integrating autoimmunity with genetic (the HLA-DR shared epitope) and environmental (smoke) risk factors for RA [12]. It has been also shown that early during disease progression aCP undergo isotype switching.
Contemporarily IgM-aCP persists during follow-up indicating that this autoimmune response is continuously reactivated during the course of arthritis [13].

4. Diagnostic and prognostic implications of RF and aCP

Despite there is no current way in clinical practice to distinguish between “natural” and RA-associated RF, the latter has a well-recognized role as clinical marker. It is part of the ACR criteria for RA and, in prognostic terms, identifies RA patients with a more severe disease functionally and radiographically, which also more frequently experience extra-articular manifestations [10,14].

ACP are very specific for RA. They are found with a significantly higher prevalence in sera of patients who will develop severe radiological damage. ACP+ patients with undifferentiated arthritis have a chance of 90% to progress to full-blown RA within 3 years [15].

In light of the growing use of new therapeutic agents in RA, these autoAbs have acquired further importance. In return, the effect of biologic treatments, and particularly of B cell depletion, expand chances to shed light into their pathogenetic role [2]. Efficacy of Rituximab has firstly been proven in RF+ patients and shown to parallel a substantial and sustained reduction of IgM-, IgG- and IgA-RF levels [16]. Also, as shown for Rituximab and anti-TNFα, RF and aCP levels decrease in response to treatment but RF reduction is more pronounced and persistent during long-term therapy suggesting a different regulation of these two autoAbs systems [17]. Moreover, it has recently been demonstrated that

Fig. 1. Lymphoid organization in RA synovium. Sections of RA synovium with a follicular pattern of infiltrating immune cells are immunostained for CD20 (A), CD3 (B), CD21 (C), CD138 (D), PNAd (E) and CXCL13 (F) antigens. Large size aggregates are characterized by a secondary lymphoid organ-like architecture, with a central area of CD20+ B cell enrichment (A) surrounded by a peripheral area of CD3+ T cells (B) and a centrally located CD21+ follicular dendritic cell network (C). CD138+ terminally differentiated plasma cells are situated at the edge of the follicle (D). The ectopic lymphoid aggregates in RA synovitis also develop a PNAd+ specialized vascular apparatus (high endothelium venules) (E) and are characterized by the in situ expression of secondary lymphoid organ chemokines such as CXCL13 (F).
high pre-treatment levels of IgA-RF are associated with poor response rate to the TNF-inhibitors in advanced RA refractory to DMARDs [18].

5. Autoantibody-independent role of B cells

5.1. Tissue architecture of synovial B cells in RA

The activation events taking place within the rheumatoid synovium are mainly dependent on local interactions and cell–cell contacts, thus assigning to tissue microarchitecture a critical importance in orchestrating immune responses.

B cells are a significant although not constant population in RA synovium. Indeed, B cell infiltration is scanty in samples lacking a defined level of organization of immune cells (diffuse synovitis) [19]. As opposed, B cells constitute a considerable fraction of the inflammatory infiltrate in samples characterized by large and well-organized mononuclear aggregates (follicular and follicular with GCs synovitis) [19]. Here, three different B cell subsets can be distinguished: terminally differentiated plasma cells that surround the follicles; mature CD20+ B cells in close interaction with CD4+ T cells; activated B cells that have a GC phenotype and proliferate in a network of follicular dendritic cells (FDCs). The enrichment of B cells in follicular structures is not synovial-specific, as it is recognized in other compartments, such as the subchondral bone marrow of involved joints [20] and the lungs [21].

Importantly, the progressive enlargement of T–B cell aggregates is paralleled by the acquisition of secondary lymphoid organ-like features (a process known as lymphoid neogenesis), such as lymphocyte compartmentalization within distinct areas, the constitution of a specialized vascular apparatus and, finally, the organization of FDC networks (Fig. 1) [19].

It is still unclear whether the above described subsets of synovial immune organization and B cell infiltration (diffuse, follicular and follicular with GCs) represent stable variants of the disease that occur in different patients, or rather reflect distinct stages and responses to therapy. Recent data seem to exclude a clear relationship between lymphocyte microarchitecture and disease activity [22]. However, the frequent occurrence of lymphoid neogenic lesions in association to tissue damage [20,21] together with the finding of a decrease in lymphoid neogenic features in patients treated with anti-TNF agents achieving clinical response [22] strongly support an association of synovial B cell aggregation with disease severity.

5.2. B cell activation and GC reaction

The establishment of a lymphoid-like architecture within the synovial lesions, with B cells in close interaction with T cells, resident cells and possibly FDCs, provides the microenvironment in which B cell activation and post-recombination processes of the BCR can occur. The analysis of the V-gene repertoire expressed in synovial B cells has demonstrated that in the inflamed synovium, besides B cell activation and oligoclonal expansion, a GC reaction can take place [5].

Different patterns of synovitis have been shown to correlate with biomarkers for B cell activity. Tissues containing GCs and B cell aggregates have the highest levels of IgG transcription if compared to samples with diffuse infiltration [23]. Furthermore, GC synovitis have increased levels of a proliferation-inducing ligand (APRIL), which is a close homolog to BLyS and acts as a modulator of peripheral B cell homeostasis promoting B cell survival and differentiation [23].

Of note, B cell activation in RA synovitis occurs both through T cell-dependent mechanisms (CD40–CD40L ligation) and T-independent pathways (BLyS, TLRs).

5.3. B cell-dependent T cell activation

Besides the effects of synovial lymphoid microarchitecture on B cell activation, the establishment of B–T cell contacts within ectopic follicles also provides the milieu in which B cells can exert their immune functions on other cells. Indeed, B cells can act as efficient APCs to stimulate T cells and to allow optimal development of memory in the CD4+ T-cell population. Compared with non-specific uptake associated with professional APCs, selective uptake of antigen by antigen-specific B cells is markedly superior. RF+ B cells, in particular, are believed to play an important role in antigen presentation [11]. They can take up antigen-Ig immune complexes via their membrane Ig receptors, which have RF specificity. B cells then process and present peptides from the antigen, and thus induce both T cell activation and T cell help [11].

An elegant demonstration that T cell response in RA synovitis is dependent on B cells and on the lymphoid organization comes from studies by Takemura and coworkers [24]. Treatment with a monoclonal anti-CD20 antibody in SCID mice transplanted with RA synovial tissue with GC formation led to disruption of GCs, loss of FDC networks and impairment of T cell activation, with fall in the production of T cell-derived cytokines.
6. B cells and cytokine production

As compared with other immune cells, B cells have not typically been considered to be a major source of cytokines. However, several lines of evidence have now established that B cells can also produce a wide spectrum of cytokines under inflammatory conditions. Notably, peripheral blood B cells from healthy subjects are a source of IL-6, TNF and lymphotoxin (LT) upon BCR and CD40 engagement [25]. There is now evidence that B cells may also play a regulatory function by modulating the production of IL-10, which can suppress harmful immune responses by regulating Th1/Th2 balance and directly dampening innate cell-mediated inflammatory responses [26].

The possible contribution of B cells in IL-10 production has been also highlighted in human RA. Indeed, cytokine profile analysis has revealed higher levels of transcription of IL-10 in follicular synovitis compared to the diffuse pattern [27].

7. B cells and ectopic lymphoid neogenesis

In RA synovial tissue B cells are the major source of LT-β, one of the members of the TNF superfamily. This cytokine plays an important role in normal lymphoid organogenesis, together with the chemokine CXCL13, which is a strong B cell attractant. Indeed, these two molecules drive the interactions between mesenchymal and hematopoietic cells in the first stages of lymphoid organ formation. Binding of LT to its receptor induces the expression of adhesion molecules and of lymphoid chemokines (CCL19, CCL21, CXCL12 and CXCL13) that regulate lymphocyte homing and compartmentalization in lymphoid tissues [28]. Similarly, transgenic expression of CXCL13 in non-lymphoid tissues is sufficient to activate a sequence of events that lead to B and T cell recruitment and segregation [29].

A reactivation of the developmental pathway driven by LT-β and CXCL13 has been thought to act up-stream the lymphoid neogenetic process in RA. Indeed, CXCL13 and LT-β have emerged as the two most important independent predictive factors in the formation of ectopic GCs in RA in a multivariate analysis [30]. Furthermore, CXCL13 production and expression strongly associate with aggregates of growing dimensions, its presence correlating in a qualitative and quantitative fashion to synovial infiltrating B cells [19]. The major cellular source of LT-β in synovial lesions has been demonstrated to be represented by a subset of mantle-zone B cells [30] while CXCL13 is produced by different cell populations [30,31].

8. Concluding remarks

B cells functions are pleiotropic in RA. They contribute to production of autoAbs, antigen presentation and cytokine production. All these functions have been dissected also through the analysis of the synovial tissue microarchitecture where B cells can find a niche to consolidate their alternative activation pathways and effectively collaborate with other resident and hematopoietic cells. Specifically treating RA with B cell-directed therapies thus means not just to deplete their compartment but also to interfere with the complex network that B cell establish with the other principal cell types known to be involved in the pathogenetic processes of RA.

Take-home messages

• B cells in RA show many features of an altered state of activation whose discover has greatly contributed to clarify the autoimmune background that leads to development of the disease.
• Autoantibodies, and mainly rheumatoid factor(s) and anti-citrullinated proteins, have been implicated in some of RA pathogenic events but also are useful biomarkers in diagnosis and prognosis and have shown correlations with response to treatment.
• It is now established that B cells have many more functions in RA than just producing autoantibodies. They are also very potent antigen-presenting cells and play a critical role in the activation of T cells. Furthermore, B cells do not only respond to but also produce cytokines.
• The cross-talk between B cells and infiltrating and resident cells in rheumatoid synovitis is guaranteed by the establishment of a complex microarchitecture that morphologically and functionally reproduces some features of secondary lymphoid organs (a process known as lymphoid neogenesis).
• B cells themselves contribute to the production of factors that orchestrate synovial lymphoid neogenesis, such as lymphotoxin.

References


